10/578263
IAP20 Rec'd PCT/PTO 04 MAY 2006

Polyvalent allergy vaccine

Description

[0001] This invention relates to novel polypeptides which are in particular suitable for permitting different allergies to be treated simultaneously. Furthermore, the present invention relates to a use of the polypeptides for producing a vaccine for treating allergic diseases.

[0002] Type 1 allergy designates the state of an immunologically induced immediate-type hypersensitivity reaction to substances (allergens) to which there is normally no sensitization.

[0003] Four types of hypersensitivity reactions are distinguished, of which types I-III are mediated by antibodies and only the type IV reaction is triggered by sensitized T cells.

[0004] The type I reaction, or also immediate-type hypersensitivity reaction, is mediated by IgE antibodies. During the sensitization phase with allergens, specific IgE antibodies are formed by B cells which arise primarily under the influence of messengers from T helper 2 cells (interleukin 4, 5, 13). Allergic persons have a genetic tendency to produce an excess of these messengers, cytokines, which can then also cause an excessively high concentration of IgE antibodies. These IgE antibodies are then bound to the Fc-ε receptors on mast cells and basophils.

[0005] Upon new contact with the allergen or allergens, the allergens are bound to the mast cell IgE and lead to a crosslinking of the IgE antibodies, thereby causing degranulation of the mast cells and basophils with release of vasoactive substances (histamine, leukotrienes, etc.). This results in the typical allergic symptoms, ranging from hay fever, conjunctivitis, bronchial asthma to anaphylactic shock.

[0006] The most important aeroallergens (there of course also being food allergens, but these lead to allergic diseases much more rarely than inhaled allergens) include tree and grass pollen, animal hair and proteins, house dust mites, latex allergens, etc.

[0007] About 20% of the population suffer from type I allergies, whereby a major portion of persons affected are sensitized not only to one allergen but to different allergens simultaneously ("polyallergic persons").

[0008] The treatment of choice (and only causal treatment) is specific immunotherapy, or SIT. Increasing doses of allergen extracts are injected into the patient so that he is hyposensitized, that is, does not react to the particular allergen as much or at all. This form of treatment can be very successful, but primarily in young and monosensitized persons, i.e. patients who are chiefly allergic to only one allergen. Further disadvantages of this treatment are that treatment lasts several years, can sometimes involve anaphylactic side effects, and treatment is done by injection, which is greatly disliked by many patients, particularly children.

[0009] Furthermore, it must be taken into account that international guidelines on immunotherapy at present warn expressly against treating polysensitized persons, primarily because of a lack of therapeutic success and an increased risk of anaphylactic side reactions during treatment.

[0010] It is known from numerous publications (Wiedermann U et al., 1999; J. Allergy Clin Immunol; 103: p 93; Garside P, 1999 Gut 44: p 137; Lowrey J et al. 1998; Int. Arch. Allergy Immunol; 116: 93) that mucosal application of a recombinant allergen can prevent allergic sensitization with the same allergen, so that both prophylaxis and therapy of an allergy can be successfully achieved.

[0011] Furthermore, there are attempts to obtain a treatment of allergies by inducing so-called blocking antibodies (usually IgG). These blocking antibodies are supposed to intercept the particular antigen/allergen so that it can no longer bind to mast cell IgE antibodies. These IgG antibodies are induced by usually employing B cell epitopes or constructs containing them.

[0012] It is known from EP 1 219 301 that hybrid allergens can be used both for treatment and for diagnosis of allergies. It is proposed here to hybridize different proteins or fragments of proteins and use these hybrid molecules for producing vaccines. Immunization with these hybrid molecules leads to formation of blocking IgE antibod-

ies. These hybrid molecules are constructed from allergens of a single allergen source and the formed antibodies are consequently aimed only to a certain allergen.

[0013] A problem of the present invention is therefore to provide novel polypeptides that permit a plurality of allergies to be treated.

[0014] Furthermore, it is a problem of the invention to provide polypeptides that permit a plurality of allergies to be treated simultaneously, in particular allergies triggered by non-cross-reacting allergens.

[0015] In addition, it is a problem of the present invention to permit a prophylaxis and/or therapy of a plurality of allergies simultaneously to be carried out preferably via the mucous membrane.

[0016] The invention is based on the finding that this can be achieved by novel hybrid polypeptides and/or chimeric allergens.

[0017] The problem is solved according to the invention by a hybrid polypeptide which comprises a multiplicity of immunodominant T cell epitopes of allergens, whereby at least two of the allergens do not cross-react with each other.

[0018] A known procedure in the treatment of allergic persons is, as mentioned above, to obtain the induction of blocking antibodies.

[0019] The basic concept of the present invention, in contrast, is that the hybrid polypeptides comprising immunodominant T cell epitopes of different allergens upon mucosal application lead to an antigen-specific nonreactivity, i.e. cause a mucosal tolerance which is obtained by deletion, anergy of antigen/allergen-specific T cells, by the production of suppressive cytokines from so-called regulatory T cells or by immunomodulation ($Th_1 > Th_2$ cells). The lack of the necessary cytokines subsequently also prevents the formation of antigen-specific antibodies. Thus, a suppression of the undesirable immune responses to the particular allergens is obtained.

[0020] A hybrid polypeptide is understood here to be a polypeptide having a multiplicity of immunodominant T cell epitopes. Preferably the hybrid polypeptide com-

prises at least five immunodominant T cell epitopes, more preferably at least four immunodominant T cell epitopes, and most preferably at least three immunodominant T cell epitopes. It is furthermore preferable that the hybrid polypeptide comprises only T cell epitopes.

[0021] An allergen is understood to be a normally harmless substance that is able to induce the formation of IgE antibodies in an allergic or atopic person and trigger immediate-type allergic symptoms (rhinitis, conjunctivitis, asthma, etc.) upon new contact. It involves complete proteins or protein fragments or peptides with amino acid sequences of different length.

[0022] An epitope is understood to be a certain region on or in the protein, usually with a length of 10-20 amino acids, which either can be recognized by specific immunoglobulins (antibodies) of B cells and/or are recognized by T cells (or their specific T cell receptors). As a rule, antibodies recognize epitopes on the tertiary structure of proteins, therefore being called B cell epitopes. These B cell epitopes correspond to either so-called conformation epitopes or linear epitopes which are situated on the surface and are readily accessible to antibodies. In contrast, the T cell epitopes which are recognized by the T cell receptors of specific T cells are always linear epitopes and situated not only on the surface of the molecule, but can be distributed randomly within the entire sequence of the molecule. The T cell epitopes only become accessible to T cells when the allergen has been taken up, processed and presented by antigen-presenting cells to the T cells or their T cell receptor by means of MHC class II molecules.

[0023] B cell epitopes and T cell epitopes usually have different localizations within the molecule, and therefore T cell epitopes are (as a rule) not recognized or bound by antibodies. This means that when an allergic person who has already formed specific antibodies is treated with a substance constituting a T cell epitope, there is no danger of these T cell epitopes being bound by the antibodies. Therefore there is also no danger of anaphylactic reactions during treatment. (This is not the case when the therapeutic substance constitutes or contains a B cell epitope.)

[0024] A further condition is that T cell epitopes of at least two allergens that do not cross-react are used. A cross reaction occurs e.g. as a reaction to antigens of related bacteria when certain subunits within their "antigen mosaic" are identical. The same can happen with proteins of related species of animals and with artificially conjugated antigens (with structural similarities of the coupled haptens).

[0025] An advantage of hybrid polypeptides from T cell epitopes that do not cross-react is that they are suitable for simultaneously treating a plurality of allergies that are not identical in their antigen mosaic.

[0026] It is preferred that all T cell epitopes of the hybrid polypeptide come from allergens that do not cross-react with each other. This provides the possibility of carrying out a treatment of multi- or polyallergic persons.

[0027] It is in particular preferred that the T cell epitopes used are selected from the series of tree pollen allergens, in particular birch allergens, preferably Bet v 1, Bet v 1 isoforms, as described in Ferreira, F et al. 1997, Int. Arch Allergy Immunol: 113;125 and Bet v 1 mutant, as described in Ferreira F et al. 1998 FASEB:12:231, grass pollen allergens (preferably Phl p 1, Phl p 2, Phl p 5, Phl p 6), latex allergens (preferably Hev b 1, Hev b 2, Hev b 3, Hev b 5, Hev b 6, Hev b 7, Hev b 8, Hev b 9, Hev b 10) and animal allergens.

[0028] It is particularly preferable that the animal allergens are animal hairs (preferably Fel d 1) and/or house dust mite allergens (preferably Der p 1, Der p 2, Der f 1, Der f 2).

[0029] It is in particular preferred that the hybrid polypeptide is a hybrid polypeptide comprising the T cell epitopes of grass pollen allergens (Phl p 1, Phl p 2, Phl p 5, Phl p 6) and/or animal allergens, in particular animal hairs (preferably Fel d 1) and/or house dust mite allergens (preferably Der p 1, Der p 2, Der f 1, Der f 2) and/or tree pollen allergens, in particular birch pollen allergens (preferably Bet v 1, Bet v 1 of isoforms or Bet v 1 of mutants).

[0030] It is even more preferred that the hybrid polypeptide is a hybrid comprising the T cell epitopes of a grass pollen allergen (preferably Phl p 1, Phl p 2, Phl p 5, Phl p 6), of a tree pollen allergen, in particular birch pollen allergen (preferably Bet v 1, Bet v 1 of isoforms or Bet v 1 of mutants) and of a latex allergen (preferably Hev b 1, Hev b 2, Hev b 3, Hev b 5, Hev b 6, Hev b 7, Hev b 8, Hev b 9, Hev b 10) and/or animal allergen. It is even more preferred that the hybrid polypeptide comprises only T cell epitopes of grass allergens (preferably Phl p 1, Phl p 2, Phl p 5, Phl p 6) and/or tree pollen allergens, in particular birch pollen allergens (preferably Ber v 1) and/or latex allergens (preferably Hev b 1, Hev b 2, Hev b 3, Hev b 5, Hev b 6, Hev b 7, Hev b 8, Hev b 9, Hev b 10) and/or animal allergens.

[0031] The hybrid polypeptide with the following amino acid sequence is particularly preferable: (sequ. 1)

MGETLLRAVESYAGELELQFRRVKCKYTVATAPEVKYTVFETALK

[0032] The problem is also solved according to the invention by providing an allergen chimera comprising at least one complete protein and at least one further allergen fragment.

[0033] An allergen chimera is understood according to the present invention to be a polypeptide comprising at least one complete protein and genetically engineered to carry at least one further allergen fragment, preferably two or three.

[0034] Such allergen chimeras are particularly suitable for treating allergies since they can be adapted individually to the allergic person.

[0035] It is particularly preferable that the protein and/or the allergen fragments, preferably protein fragments, do not cross-react with each other. It is in particular preferable that the protein and the integrated allergen fragments, preferably protein fragments, all do not cross-react with each other. The advantage of such an allergen chimera is that a polyallergic person can be treated for different allergies simultaneously, also for ones that do not cross-react with each other.

[0036] Furthermore, it is preferred that the allergen fragments, preferably protein fragments, are T cell epitopes. The advantage of such an allergen chimera is that, as described above, the production of IgG antibodies is not activated, but a suppression of the production of IgE antibodies occurs by deactivation of cytokine synthesis. A further advantage of an allergen chimera is that it is a molecule with a tertiary structure which can be taken up with increased efficiency by antigen-presenting cells (e.g. dendritic cells, B cells, etc.), can be processed, and thus the immunodominant peptides can be presented to the specific T cells. The use of molecules with a tertiary structure furthermore permits the tolerogenic effect to be increased.

[0037] It is in particular preferred that the complete protein in the allergen chimera is a protein selected from the group of tree pollen allergens, in particular birch allergens (preferably Bet v 1, Bet v 1 of isoforms or Bet v 1 of mutants), grass pollen allergens (preferably Phl p 1, Phl p 2, Phl p 5, Phl p 6), latex allergens (preferably Hev b 1, Hev b 2, Hev b 3, Hev b 5, Hev b 6, Hev b 7, Hev b 8, Hev b 9, Hev b 10) and animal allergens, in particular animal hairs (preferably Fel d 1) and/or house dust mite allergens (preferably Der p 1, Der p 2, Der f 1, Der f 2). It is in particular preferable that the protein is a tree pollen allergen, in particular a birch allergen, and particularly preferably the protein Bet v 1, Bet v 1 of isoforms or Bet v 1 of mutants.

[0038] Furthermore, it is preferred that the allergen fragments are T cell epitopes selected from the group comprising grass pollen allergens (preferably Phl p 1, Phl p 2, Phl p 5, Phl p 6), latex allergens (preferably Hev b 1, Hev b 2, Hev b 3, Hev b 4, Hev b 5, Hev b 6, Hev b 7, Hev b 8, Hev b 9, Hev b 10) and animal allergens, in particular animal hairs (preferably Fel d 1) and/or house dust mite allergens (preferably Der p 1, Der p 2, Der f 1, Der f 2).

[0039] It is in particular preferred that the allergen fragments, preferably protein fragments, are T cell epitopes of grass pollen allergens (preferably Phl p 1, Phl p 2, Phl p 5, Phl p 6) and/or latex allergens (preferably Hev b 1, Hev b 2, Hev b 3, Hev b 5, Hev b 6, Hev b 7, Hev b 8, Hev b 9, Hev b 10) and/or animal allergens, in particular animal hairs (preferably Fel d 1) and/or house dust mite allergens (preferably Der p 1,

Der p 2, Der f 1, Der f 2), and the complete protein is Bet v 1, Bet v 1 of isoforms or Bet v 1 of mutants.

[0040] It is in particular preferred that the allergen chimera constructs comprise Bet v 1, Bet v 1 of isoforms or Bet v 1 of mutants and the immunodominant peptides of Phl p 1 and Phl p 5. The following constructs are most preferred:

Construct 1 (Phl p 1 – Bet v 1a – Phl p 5) – amino acid sequence (sequ. 2)

MEQKLRSAGELELQFRRVKCKYPEGTKVEFGVFNYETETTSVIPAARLK
AFILDGDNLFPKVAPQAINIEGNGGPGTKISPEGFPFKYVKDRVDEVDHT
NFKYNYSVIEGGPIGDTLEKISNEIKIVATPDGGSILKISNKYHTKGDHEVK
AEQVKASKEGETLRVESYLLAHSDAYNKLQAYAATVATAPEVKYTVFE
TALKKATAMSE

Construct 2 (Phl p 5 – Bet v 1a – Phl p 1) – amino acid sequence (sequ. 3)

MAYAATVATAPEVKYTVFETALKKAITAMSEEFGVFNYETETTSVIPAA
RLFKAFILDGDNLFPKVAPQAISSVENIEGNGGPGTIKKISFPEGFPFKYVK
DRVDEVDHTNFKYNYSVIEGGPIGDTLEKISNEIKIVATPDGGSILKISNKY
HTKGDHEVKAEQVKASKEMGETLLRAVESYLLAHSDAYNKLEQKLRSA
GELELQFRRVKCKYPEGTKV

Construct 3 (Bet v 1a, Phl p 1, Phl p 5) – amino acid sequence (sequ. 4)

MGEFGVFNYETETTSVIPAARLFKAFILDGDNLFPKVAPQAISSVENIEGN
GGPGTIKKISFPEGFPFKYVKDRVDEVDHTNFKYNYSVIEGGPIGDTLEKI
SNEIKIVATPDGGSILKISNKYHTKGDHEVKAEQVKASKEMGETLLRAVE
SYLLAHSDAYNKLEQKLRSAGELELQFRRVKCKYPEGTKVTSQAYAAT
VATAPEVKYTVFETALKKAITAMSE

Construct 4 (Phl p 1 – Phl p 5 – Bet v 1a) – amino acid sequence (sequ. 5)

MEQKLRSAGELELQFRRVKCKYPEGTKVTSQAYAATVATAPEVKYTVF
ETALKKAITAMSEEFGVFNYETETTSVIPAARLFKAFILDGDNLFPKVAPQ
AISSVENIEGNGGPGTIKKISFPEGFPFKYVKDRVDEVDHTNFKYNYSVIE

GGPIGDTLEKISNEIKIVATPDGGSILKISNKYHTKGDHEVKAEQVKASKE
MGETLLRAVESYLLAHSDAYN

[0041] The invention furthermore comprises polynucleotides encoding the allergen chimera according to the present invention. By the degeneration of the genetic code, different polynucleotide molecules can code for a single allergen chimera. The polynucleotides of the present invention are preferably an expression construct in order to obtain the polypeptides after expression in the host cell. The expression construct can contain further components which are necessary for expression and belong to general prior art, such as promoter sequences, gene coding resistance factors against certain antibiotics as well as a replication origin.

<u>Construct 1 (Phl p 1 – Bet v 1a – Phl p 5) – nucleotide sequence</u> (sequ. 6)

CC ATG GAG CAG AAG CTG CGC AGC GCC GGC GAG CTG GAG CTC CAG
TTC CGG CGC GTC AAG TGC AAG TAC CCG GAG GGC ACC AAG GTG GAA
TTC GGT GTT TTC AAT TAC GAA ACT GAG ACC ACC TCT GTT ATC CCA
GCA GCT CGA CTG TTC AAG GCC TTT ATC CTT GAT GGC GAT AAT CTC
TTT CCA AAG GTT GCA CCC CAA GCC ATT AGC AGT GTT GAA AAC ATT
GAA GGA AAT GGA GGG CCT GGA ACC ATT AAG AAG ATC AGC TTT CCC
GAA GGC TTC CCT TTC AAG TAC GTG AAG GAC AGA GTT GAT GAG GTG
GAC CAC ACA AAC TTC AAA TAC AAT TAC AGC GTG ATC GAG GGC GGT
CCC ATA GGC GAC ACA TGG AGA AGA TCT CC AAC GAG ATA AAG ATA
GTG GCA ACC CCT GAT GGA GGA TCC ATC TTG AAG ATC AGC AAC AAG
TAC CAC ACC AAA GGT GAC CAT GAG GTG AAG GCA GAG CAG GTT AAG
GCA AGT AAA GAA ATG GGC GAG ACA CTT TTG AGG GCC GTT GAG AGC
TAC CTC TTG GCA CAC TCC GAT GCC TAC AAC AAG CTT CAG GCC TAC
GCC GCC ACC GTC GCC ACC GCG CCG GAG GTC AAG TAC ACT GTC TTT
GAG ACC GCA CTG AAA AAG GCC ATC ACC GCC ATG TCC GAA TAA CTC
GAG

Construct 2 (Phl p 5 – Bet v 1a – Phl p 1) – nucleotide sequence (sequ. 7)

CC ATG GCC TAC GCC GCC ACC GTC GCC ACC GCG CCG GAG GTC AAG
TAC ACT GTC TTT GAG ACC GCA CTG AAA AAG GCC ATC ACC GCC ATG
TCC GAA GAA TTC GGT GTT TTC AAT TAC GAA ACT GAG ACC ACC TCT
GTT ATC CCA GCA GCT CGA CTG TTC AAG GCC TTT ATC CTT GAT GGC
GAT AAT CTC TTT CCA AAG GTT GCA CCC CAA GCC ATT AGC AGT GTT
GAA AAC ATT GAA GGA AAT GGA GGG CCT GGA ACC ATT AAG AAG
ATC AGC TTT CCC GAA GGC TTC CCT TTC AAG TAC GTG AAG GAC AGA
GTT GAT GAG GTG GAC CAC ACA AAC TTC AAA TAC AAT TAC AGC GTG
ATC GAG GGC GGT CCC ATA GGC GAC ACA TGG AGA AGA TCT CC AAC
GAG ATA AAG ATA GTG GCA ACC CCT GAT GGA GGA TCC ATC TTG AAG
ATC AGC AAC AAG TAC CAC ACC AAA GGT GAC CAT GAG GTG AAG GCA
GAG CAG GTT AAG GCA AGT AAA GAA ATG GGC GAG ACA CTT TTG AGG
GCC GTT GAG AGC TAC CTC TTG GCA CAC TCC GAT GCC TAC AAC AAG
CTT GAG CAG AAG CTG CGC AGC GCC GGC GAG CTG GAG CTC CAG TTC
CGG CGC GTC AAG TGC AAG TAC CCG GAG GGC ACC AAG GTG TAA CTC
GAG

Construct 3 (Bet v 1a – Phl p 1 – Phl p 5) – nucleotide sequence (sequ. 8)

CC ATG GGA GAA TTC GGT GTT TTC AAT TAC GAA ACT GAG ACC ACC
TCT GTT ATC CCA GCA GCT CGA CTG TTC AAG GCC TTT ATC CTT GAT
GGC GAT AAT CTC TTT CCA AAG GTT GCA CCC CAA GCC ATT AGC AGT
GTT GAA AAC ATT GAA GGA AAT GGA GGG CCT GGA ACC ATT AAG AAG
ATC AGC TTT CCC GAA GGC TTC CCT TTC AAG TAC GTG AAG GAC AGA
GTT GAT GAG GTG GAC CAC ACA AAC TTC AAA TAC AAT TAC AGC GTG
ATC GAG GGC GGT CCC ATA GGC GAC ACA TGG AGA AGA TCT CC AAC
GAG ATA AAG ATA GTG GCA ACC CCT GAT GGA GGA TCC ATC TTG AAG
ATC AGC AAC AAG TAC CAC ACC AAA GGT GAC CAT GAG GTG AAG GCA
GAG CAG GTT AAG GCA AGT AAA GAA ATG GGC GAG ACA CTT TTG AGG
GCC GTT GAG AGC TAC CTC TTG GCA CAC TCC GAT GCC TAC AAC AAG
CTT GAG CAG AAG CTG CGC AGC GCC GGC GAG CTG GAG CTC CAG TTC

CGG CGC GTC AAG TGC AAG TAC CCG GAG GGC ACC AAG GTG ACT AGT CAG GCC TAC GCC GCC ACC GTC GCC ACC GCG CCG GAG GTC AAG TAC ACT GTC TTT GAG ACC GCA CTG AAA AAG GCC ATC ACC GCC ATG TCC GAA TAA CTC GAG

Construct 4 (Phl p 1 – Phl p 5 – Bet v 1a) – nucleotide sequence (sequ. 9)

CC ATG GAG CAG AAG CTG CGC AGC GCC GGC GAG CTG GAG CTC CAG TTC CGG CGC GTC AAG TGC AAG TAC CCG GAG GGC ACC AAG GTG ACT AGT CAG GCC TAC GCC GCC ACC GTC GCC ACC GCG CCG GAG GTC AAG TAC ACT GTC TTT GAG ACC GCA CTG AAA AAG GCC ATC ACC GCC ATG TCC GAA GAA TTC GGT GTT TTC AAT TAC GAA ACT GAG ACC ACC TCT GTT ATC CCA GCA GCT CGA CTG TTC AAG GCC TTT ATC CTT GAT GGC GAT AAT CTC TTT CCA AAG GTT GCA CCC CAA GCC ATT AGC AGT GTT GAA AAC ATT GAA GGA AAT GGA GGG CCT GGA ACC ATT AAG AAG ATC AGC TTT CCC GAA GGC TTC CCT TTC AAG TAC GTG AAG GAC AGA GTT GAT GAG GTG GAC CAC ACA AAC TTC AAA TAC AAT TAC AGC GTG ATC GAG GGC GGT CCC ATA GGC GAC ACA TGG AGA AGA TCT CC AAC GAG ATA AAG ATA GTG GCA ACC CCT GAT GGA GGA TCC ATC TTG AAG ATC AGC AAC AAG TAC CAC ACC AAA GGT GAC CAT GAG GTG AAG GCA GAG CAG GTT AAG GCA AGT AAA GAA ATG GGC GAG ACA CTT TTG AGG GCC GTT GAG AGC TAC CTC TTG GCA CAC TCC GAT GCC TAC AAC TAA CTC GAG

[0042] Furthermore, the invention comprises a pharmaceutical composition comprising a hybrid polypeptide and/or an allergen chimera as described above.

[0043] In particular, this is a vaccine composition comprising at least a hybrid polypeptide and/or an allergen chimera as described above which must be soluble. Upon application the allergen chimera is dissolved in physiological saline solution and applied.

[0044] Furthermore, it is preferred that the composition comprising the hybrid polypeptide and/or the allergen chimera as described above is suitable for treating an allergic disease.

[0045] Furthermore, it is preferred that the pharmaceutical composition, in particular vaccine, comprises a mucosal adjuvant and/or antigen transport system, such as lactic acid bacteria.

[0046] Certain lactic acid bacteria have the property of inducing a Th1 immune response. For this purpose it is expedient to use lactic acid bacteria as an expression system for producing proteins and peptides. As a mucosal (oral) vaccine, such lactic acid bacteria can influence the immune response such that allergic reactions can be prevented or modulated. Therefore, the vaccine preferably additionally comprises lactic acid bacteria in order to improve the action upon application of hybrid peptides or allergen chimeras.

[0047] Further, the invention comprises the use of a hybrid polypeptide and/or an allergen chimera as described above for producing a drug, in particular a vaccine. It is furthermore preferred that the use of a hybrid polypeptide and/or allergen chimera serves to produce a vaccine for treating allergies.

[0048] Further, it is preferred that the hybrid polypeptide and/or the allergen chimera as described above is used for producing a vaccine for simultaneous treatment of at least two different allergies.

[0049] Furthermore, it is preferred that the hybrid polypeptide and/or the allergen chimera is used for producing a vaccine for simultaneous treatment of at least two different allergies, the latter being triggered by non-cross-reacting allergens.

[0050] In particular, it is preferred that upon use of the hybrid polypeptides and/or allergen chimeras for producing a vaccine, these hybrid polypeptides have fragments of at least two non-cross-reacting allergens, and/or the allergen chimeras have proteins and fragments of at least two non-cross-reacting allergens.

[0051] Furthermore, it is preferred that upon use of the hybrid polypeptides and/or allergen chimeras for producing a vaccine, hybrid polypeptides comprise only fragments and/or the allergen chimeras only proteins and fragments that do not cross-react with each other.

[0052] It is furthermore preferred that upon use of the hybrid polypeptides and/or allergen chimeras for producing a vaccine, the hybrid polypeptides or the allergen chimeras have T cell epitopes of the allergens.

[0053] Furthermore, it is preferred that upon use of hybrid polypeptides and/or allergen chimeras for producing a vaccine, the allergen fragments are T cell epitopes selected from the series comprising grass pollen allergens (preferably Phl p 1, Phl p 2, Phl p 5, Phl p 6), tree pollen allergens, in particular birch pollen allergens (preferably Bet v 1, Bet v 1 of isoforms or Bet v 1 of mutants), latex allergens (preferably Hev b 1, Hev b 2, Hev b 3, Hev b 5, Hev b 6, Hev b 7, Hev b 8, Hev b 9, Hev b 10) and animal allergens, in particular animal hairs (preferably Fel d 1) and/or house dust mite allergens (preferably Der p 1, Der p 2, Der f 1, Der f 2).

[0054] Furthermore, it is preferred that upon use of allergen chimeras for producing a vaccine, the protein is a protein selected from the group comprising grass pollen allergens (preferably Phl p 1, Phl p 2, Phl p 5, Phl p 6), tree pollen allergens, in particular birch pollen allergens (Bet v 1, Bet v 1 of isoforms or Bet v 1 of mutants), latex allergens (preferably Hev b 1, Hev b 2, Hev b 3, Hev b 5, Hev b 6, Hev b 7, Hev b 8, Hev b 9, Hev b 10) and animal allergens, in particular animal hairs (preferably Fel d 1) and/or house dust mite allergens (preferably Der p 1, Der p 2, Der f 1, Der f 2).

[0055] Furthermore, hybrid polypeptides and/or allergen chimeras as defined above can be used for producing a vaccine for treating allergies, in particular for treating allergies based on two non-cross-reacting allergens.

[0056] Furthermore, it is preferred that the hybrid polypeptides and/or allergen chimeras are used for producing a vaccine such that it can be used for prophylaxis and/or therapy of allergies based on at least two non-cross-reacting allergens.

[0057] Furthermore, it is preferred that the hybrid polypeptides and/or the allergen chimeras are used for producing a vaccine such that the vaccine can be administered nasally, rectally or orally. However, it is also conceivable that the hybrid polypeptides and/or the allergen chimeras are used for producing a vaccine such that the vaccine can be used for systemic treatment. It is preferred, however, that administration can be done nasally, rectally or orally, particularly preferably nasally.

[0058] Furthermore, it is preferred that the use of the pharmaceutical composition, in particular vaccine, comprises a mucosal adjuvant and/or antigen transport system, such as lactic acid bacteria.

[0059] The vaccines described in the method section are particularly preferred.

[0060] It is preferred that the vaccine has a dose of hybrid polypeptide and/or allergen chimera at least of 20 μg.

[0061] It is furthermore preferred that the vaccine is so constructed that it can be applied at least three times at a one-week interval. It is preferred, however, that administration can be done nasally, rectally or orally, particularly preferably nasally.

[0062] Furthermore, the invention also comprises the method for producing hybrid polypeptides and the methods for producing allergen chimeras.

[0063] The hybrid polypeptides are preferably produced by chemical synthesis, particularly preferably by the peptide synthesis stated in the methods section.

[0064] The allergen chimeras are preferably produced by recombination technology. This is done using polynucleotides coding for the allergen chimera, these polynucleotides being inserted into a host cell and this host cell being cultured under certain conditions so that the allergen chimera is expressed. Then the expression product is separated from the cell. The polynucleotides can be produced by known methods or it is preferred that PCR technology is used to produce polynucleotides encoding the allergen chimeras.

[0065] The invention will furthermore be illustrated more exactly by the following examples, without being limited thereto.

Material and methods

Animals

[0066] 7-week-old female BALB/c mice were obtained from Charles River (Sulzfeld, Germany). All experiments were approved by the Commission on Animal Experimentation of the University of Vienna and the Ministry of Development, Research and Culture.

Recombinant allergens, natural allergen extracts

[0067] Bet v 1, Phl p 1 and Phl p 5 were obtained from Biomay GmbH (Linz, Austria). Birch pollen and pollen of Phleum pratense (timothy grass) were obtained from Allergon (Välinge, Sweden) and the extracts thereof produced according to Wiedermann et al., 1999 (J. Allergy Clin. Immunol. 103:1202).

Synthesis, purification and characterization of peptides

[0068] The peptides were synthesized by using an Fmoc (9-fluorenyl methoxycarbonyl) method with HBTU (2-(1-H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) activation (0.1 mmol small cycles) on an Applied Biosystems (Foster City, CA) peptide synthesizer model 433A. Preloaded PEG-PS (polyethyleneglycol-polystyrene resins (0.15-0.2 mmol/g filler; from Septive Biosystems, Warrington, UK) were used as the solid phase to form the peptides. The chemical materials were purchased from PE Applied Biosystems. The coupling of amino acids was monitored by measuring the conductivity with feedback regulation. The peptides were separated from the resin with the following solution: 2 hours 250 µl distilled water, 250 µl triisopropylsilane (Fluka, Buchs, Switzerland), and 9.5 ml trifluoroacetic acid and precipitated in tert-butyl methyl ether (Fluka, Buchs, Switzerland). The identity of the peptides was checked by mass spectrometry and the peptides purified to >90% purity by preparative HPLC (piCHEM, Graz, Austria).

Production/clonation of allergen chimeras:

[0069] First, expression plasmids containing cDNA coding for Bet v 1a are produced in the vector pHis-parallel 2 (Table 1). The pHis-parallel 2 contains a T7 promoter for expression induction by IPTG (isopropyl-beta-D-thiogalactopyranoside), followed by an N-terminal 6x-histidine tag and an interface for TEV (tobacco etch viral protease) by which the His tag can be separated. Using the 6x-His tag the expressed allergen can be purified via Ni-NTA (nickel-nitrilotriacetic acid) agarose (Qiagen).

[0070] The primers used are 5a and 5b for constructs 1-3 and 5a and 5c for construct 4 (with terminal Bet v 1a). The primers each contain an interface for the vector (Nco I or Xho I), an interface for inserting further cDNA (Eco R I or Hind III), or a stop codon (5c) and Bet v 1a partial sequences. PCR is used to amplify the cDNA of Bet v 1 which also contains start and stop codons. After purification and digestion with the corresponding restriction enzymes (Nco I or Xho I) the construct is inserted into the pHis-parallel vector (see Table 1).

[0071] In further steps the Phl p 1 and Phl p 5 fragments are amplified with the corresponding primers (1a-d, 2a-d, 3a-d, 4a-d) which likewise contain interface and sequence parts. After purification and enzyme digestion the fragments are inserted into the vector which already contains the Bet v 1a sequence. Using the two interfaces, at the 3' end and 5' end in each case, it is possible to insert in future as different and as many allergen fragments as desired (e.g. immunodominant peptides of latex allergens, house dust, cat allergens, etc.).

[0072] The thus constructed plasmids are transformed into BL21 (DE3) cells, an E. Coli strain, selected via LB-Amp (100 mg/l ampicillin) plates and a single colony is chosen. For protein expression this clone is grown in liquid LB-Amp medium. Expression is then induced by means of 1 mM IPTG. Cell disruption and protein purification by Ni-NTA are carried out by established protocols (Qiagen).

Table 1: Primers

Construct 1: Pept 2 – Bet v 1a – Pept 4

(1a) 5'-primer Phl p 1 fwd Nco (sequ. 10)
5' CATGCCATGGAGCAGAAGCTGCGCAGC 3'

(1b) 3'-primer Phl p 1 rev Eco (sequ. 11)
5' ATGAATTCCACCTTGGTGCCCTCCGG 3'

(1c) 5'-primer Phl p 5 fwd Hind (sequ. 12)
5' ACCAAGCTTCAGGCCTACGCCGCCACC 3'

(1d) 3'-primer Phl p 5 rev Xho stop (sequ. 13)
5' CCGCTCGAGTTATTCGGACATGGCGGTGAT 3'

Construct 2: Pept 4 – Bet v 1a – Pept 2

(2a) 5'-primer Phl p 1 fwd Hind (sequ. 14)
ACCAAGCTTGAGCAGAAGCTGCGCAGC

(2b) 3'-primer Phl p 1 rev Xho stop (sequ. 15)
5' CCGCTCGAGTTACACCTTGGTGCCCTCCGG 3'

(2c) 5'-primer Phl p 5 fwd Nco (sequ. 16)
5' CATGCCATGGCCTACGCCGCCACCGTC 3'

(2d) 3'-primer Phl p 5 rev Eco (sequ. 17)
5' ATGAATTCTTCGGACATGGCGGTGAT 3'

Construct 3: Bet v 1a – Pept 2 – Pept 4

(3a) 5'-primer Phl p 1 fwd Hind (sequ. 18)
5' ACCAAGCTTGAGCAGAAGCTGCGCAGC 3'

(3b) 3'-primer Phl p 1 rev Spe (sequ. 19)
5' GGACTAGTCACCTTGGTG000TCCGG 3'

(3c) 5'-primer Phl p 5 fwd Spe (sequ. 20)
5' GGACTAGTCAGGCCTACGCCGCCACC 3'

(3d) 3'-primer Phl p 5 rev Xho stop (sequ. 21)
5' CCGCTCGAGTTATTCGGACATGGCGGTGAT 3'

<u>Construct 4: Pept 2 – Pept 4 – Bet v 1a</u>

(4a) 5'-primer Phl p 1 fwd Nco (sequ. 22)
5' CATGCCATGGAGCAGAAGCTGCGCAGC 3'

(4b) 3'-primer Phl p 1 rev Spe (sequ. 23)
5' GGACTAGTCACCTTGGTGCCCTCCGGG 3'

(4c) 5'-primer Phl p 5 fwd Spe (sequ. 24)
5' GGACTAGTCAGGCCTACGCCGCCACC 3'

(4d) 3'-primer Phl p 5 rev Eco (sequ. 25)
5' ATGAATTCTTCGGACATGGCGGTGAT 3'

<u>Bet v 1a</u>

(5a) 5' primer Bet start Nco Eco (sequ. 26)
5' CATGCCATGGGAGAATTCGGTGTTTTCAATTACGAAACTG 3'

(5b) 3' primer Bet stop Hind Xho (sequ. 27)
5' CCGCTCGAGTCCAAGCTTGTTGTAGGCATCGGAGTGTG 3'

(5c) 3' primer Bet stop +stop Xho (sequ. 28)
5' CCGCTCGAGTTAGTTGTAGGCATCGGAGTG 3'

Sensitization

[0073] Sensitization was effected by 3 injections of a mixture of 5 µg Bet v 1, 5 µg Phl p 1 and 5 µg Phl p 5 adsorbed on $Al(OH)_3$ into the abdominal cavity at a time interval of 14 days. Polysensitization was carried out (Group 1, n=5). Sampling and analysis were carried out seven days after the last immunization.

Tolerance induction

[0074] For tolerance induction, a mixture of 3 allergens (10 µg each) was administered intranasally (i.n.) three times at a time interval of seven days before polysensitization (Group 2, n=5). For peptide-induced tolerance, a mixture of 5 µg Bet v 1 peptide, 5 µg Phl p 1 peptide and 5 µg each of the two Phl p 5 (Group 3, n=5) or 20 µg hybrid peptides as described above were used. The sensitized control mice were placebo-treated by being given 30 µl of 0.9% NaCl intranasally. Sampling and analysis were carried out seven days after the last immunization.

Cutaneous type 1 hypersensitization reaction

[0075] Seven days after the last immunization, intradermal skin tests were carried out. 100 microliters of 0.5% Evans Blue (Sigma, St. Louis, Mo) was injected intravenously into the tail veins of the mice. Thereupon 30 µl Bet v 1 or Phl p 1 or Phl p 5 (2.5 µg/ml) were injected intradermally into the shaved abdominal skin. The mast cell degranulating substance 48/80 (20 µg/ml; Sigma) served as a positive control and PBS was used as a negative control. After 20 minutes the mice were killed and the color intensity of the reaction was compared with the individual positive control on the inner side of the abdominal skin.

Sampling

[0076] Blood samples were taken from the tail veins before and seven days after sensitization, the serum was recovered and stored at -20°C until analysis. Spleens were removed under sterile conditions. The organs were homogenized and filtered through sterile filters. The erythrocytes were lysed and resuspended in a cell medium (RPMI, 10% FCS, 0.1 mg/ml gentamicin, 2 mmol/l glutamine and 50 µmol/l 2-mercaptoethanol).

Detection of allergen-specific antibodies in serum

[0077] Microtiter plates (Nunc) were coated overnight at 4°C with Bet v 1 (5 µg/ml), Phl p 1 (5 µg/ml) or Phl p 5 (5 µg/ml). After washing and blocking with 1% BSA-PBS/Tween, serum samples were diluted 1/1,000 for IgG1 antibodies, 1/500 for

IgG2a antibodies and 1/10 for IgE antibodies. Rat anti-mouse IgG1, IgG2a and IgE antibodies (1/500, Pharmingen, San Diego, California) and subsequently peroxidase-conjugated mouse anti-rat IgG antibodies (1/2000, Jackson Immuno Lab, West Grove, PA) were used. The color development was carried out as described earlier (Wiedermann et al. 1999). The results show the ODs after subtraction of the basic values of preimmune sera.

Lymphocyte proliferation assay

[0078] The spleen cell suspensions were plated out at a concentration of $2x10^5$ cells/well on 96 plates (Nunc, Roshilde, Denmark) and stimulated for 4 days both with and without concanavalin A (Con A; 0.5 μg/well; Sigma), Bet v 1 (2 μg/well), Phl p 1 (2 μg/well) or Phl p 5 (2 μg/plate). Then the cultures were incubated with 0.5 μCi/well ^{3}H-thymidine (Amersham, Buckinghamshire, UK) for 16 hours. Proliferation was measured by scintillation counting. The ratio of proliferation after antigen stimulation (cpm) to proliferation after addition of medium (cpm) was determined (stimulation index (SI)).

Epitope mapping

[0079] To permit the immunodominant peptides of Bet v 1, Phl p 1 and Phl p 5 to be localized, T cell epitope mapping was carried out. Use was made of dodecapeptides overlapping in 3 amino acids in each case which span the entire sequence of the individual proteins. 50 Bet v 1 peptides, 77 Phl p 1 peptides and 92 Phl p 5 peptides were incubated with spleen cells of immunized mice, and the proliferation rates after incorporation of ^{3}H-thymidine measured by means of a beta counter.

Measurement of cytokine production

[0080] For determining the IFN-γ, IL-4, IL-5 and IL-10 production, the spleen cell suspension was cultured with or without Con A (2.5 μg/plate), birch pollen (25 μg/plate) or Phleum extract (25 μg/plate) in 48 plates (Costar, Cambridge, Mass.) at a concentration $5x10^6$ cells/plate. 40 hours later the supernatant was removed and stored at -20°C until analysis.

[0081] IL-4 and IL-10 concentrations were measured with mouse ELISA kits (Endogen, Cambridge, Mass.). IFN-γ concentrations were measured as follows: supernatants were applied undiluted to ELISA plates coated with anti-mouse IFN-γ. Then biotin-conjugated rat anti-mouse IFN-γ antibodies (0.1 μg/ml, Endogen) were applied, followed by peroxidase-conjugated streptavidin (1:10 000 in PBS/4% bovine serum albumen (BSA); Endogen). Cytokine concentration was within the pg/ml range.

Results

Characterization of immune responses to Bet v 1, Phl p 1 and Phl p 5 in polysensitized mice

[0082] Allergen-specific antibodies and type I skin tests: Polysensitized mice showed high IgG1 and IgE antibody fractions against all three antigens. Bet v 1- and Phl p 1-specific IgG2a antibody concentrations were lower in comparison with Phl p 5-specific IgG2a production (Fig. 1). In accordance with the increased IgG1/IgE antibody concentration, all polysensitized mice showed strong type I skin reactions to Bet v 1, Phl p 1 and Phl p 5 in vivo.

[0083] Lymphoproliferation of spleen cells and cytokine production in vitro: A strong lymphoproliferation was observed after stimulation with all 3 antigens. T cell proliferation (SI) was strongest after stimulation with Phl p 5 and comparably strong after stimulation with Bet v 1 and Phl p 1 (Table 2). Additionally, a pronounced IL-4, IL-5 and IFN-γ production was ascertained after the spleen cell suspension from polysensitized mice was stimulated with birch pollen and Phleum extract (Table 2). Naive splenocytes treated with Bet v 1, Phl p 1, Phl p 5, BP or Phleum extract did not differ in their proliferation response or in cytokine content from those of the control medium, which proves the antigen-specific response of spleen cell cultures from immunized mice (data not listed).

[0084] Epitope mapping: To ascertain reactive T cell epitopes of Bet v 1, Phl p 1 and Phl p 5, the spleen cell suspension from polysensitized mice was stimulated with the corresponding peptides.

[0085] For Bet v 1 (accession number P15494) these experiments showed an immunodominant T cell epitope MGETLLRAVESY at the C-terminus, corresponding to position 139-150 of the amino acid sequence, which was described and published by Bauer et al. Further, the same epitope was identified as an immunodominant peptide in patients allergic to birch pollen (Ebner et al.).

[0086] For Phl p 1 (accession number P43213) an immunodominant region AGELELQFRRVKCKY was identified, which corresponds to position 127-141 of the amino acid sequence. These epitopes were also described as T cell-reactive regions in human in vitro studies with Phl p 1-specific T cell lines and T cell clones (Schenk, Ebner). With regard to Phl p 5 (accession number Q40960), two immunodominant regions were identified, namely KVDAAFKVAATAANA, which corresponds to the amino acid sequence 166-180, and TVATAPEVKYTVFETALK, which corresponds to the amino acid sequence 226-243. The same sequences were previously described in human in vitro studies with Phl p 5-specific T cell lines and T cell clones in patients allergic to grasses (Müller), which proves the clinical importance of this animal model.

[0087] These results were the basis for the synthesis of allergen-specific peptides and of a hybrid peptide comprising the immunodominant epitopes of Bet v 1, Phl p 1 and Phl p 5 (Table 3) . Consequently we compared the effectiveness of a combination of the three allergens, a mixture of the immunodominant peptides or the hybrid peptide to induce a mucosal tolerance for preventing polysensitization.

Table 2:

T cell proliferation and cytokine production in spleen cell cultures from immunized mice with a composition of Bet v 1/Phl p 5 adsorbed on $Al(OH)_3$

	Bet v 1	**Phl p 1**	**Phl p 5**
SI	2.97 ± 0.51	2.63 ± 1.02	4.41 ± 2.38
	IFN-γ (pg/ml)	**IL-4 (pg/ml)**	**IL-5 (pg/ml)**
BP	1933.3 ± 901.6	31.1 ± 16.59	51.7 ± 42.52
Phleum extract	1333.3 ± 288.7	41.2 ± 8.62	246.7 ± 105.1

[0088] Spleen cells of immunized mice with a composition of Bet v 1/Phl p 1/Phl p 5 were grown with the corresponding antigens. A proliferation response was measured by ^3H incorporation and stated as the stimulation index (SI). IFN-γ, IL-4 and IL-5 concentrations were measured by ELISA in supernatants after 40 hours of stimulation with birch pollen (BP) or Phleum extract. All results are average values (± SD) from three independent experiments with five animals per experiment.

Table 3:

PEPTIDES

Bet v 1 (no. 47):	SKEMGETLLRAVESYLLAHSDE
Phl p 1 (no. 43/44):	LRSAGELELQFRRVKCKYPEG
Phl p 5/1 (no. 56/57):	VIEKVDAAFKVAATAANAAPANDK
Phl p 5/2 (no. 76/78):	YAATVATAPEVKYTVFETALKKAI

HYBRID PEPTIDE

```
1          12              27               45
MGETLLRAVESYAGELELQFRRVKCKYTVATAPEVKYTVFETALK
```

AA 1-12	Bet v 1 (no. 47)
AA 13-27	Phl p 1 (no. 43/44)
AA 28-45	Phl p 5/2 (no. 76/78)

Induction of mucosal tolerance by intranasal coapplication of Bet v 1, Phl p 1 and Phl p 5

[0089] Allergen-specific antibody concentrations, lymphoproliferation response of spleen cells and cytokine production in vivo: The coapplication of a mixture of Bet v 1, Phl p 1 and Phl p 5 before polysensitization led to a decrease of the Bet v 1-specific IgG1, IgE and IgG2a concentration in comparison with polysensitized control animals. In contrast, Phl p 1- and Phl p 5-specific antibody production tended to increase, especially Phl p 5-specific IgG2a concentration (data not shown). No significant effect was shown regarding the proliferation of spleen cells or allergen-specific cytokine production in vitro (data not shown). Therefore, tolerance induction was carried out with a

mixture of immunodominant peptides or a hybrid peptide with immunodominant peptides of all three allergens.

Tolerance induction with mixture of immunodominant peptides in comparison to hybrid polypeptide

[0090] Allergen-specific antibody concentration: Intranasal pretreatment with the peptide mixture as well as with the hybrid peptide reduced Phl p 1-specific, but not Bet v 1- or Phl p 5-specific, IgG1 production (Table 4). In contrast, IgG2a concentration increased substantially in mice treated with the peptide mixture (Table 4A), simultaneously the IgE/IgG2a ratio was reduced for Bet v 1- and Phl p 1-specific antibodies by 60% and by 30% for the Phl p 5-specific antibodies in polytolerized mice. Upon intranasal application of the hybrid peptide, similar results were achieved regarding the IgG2a antibody production (Table 4B). However, this pretreatment led to an 80% decrease in IgE/IgG2a concentration in Bet v 1-specific antibody concentrations and to an 80% decrease in Phl p 5-specific antibody concentrations in comparison with polysensitized control animals (Figure 2B).

Table 4: Allergen-specific antibodies in polysensitized sera in comparison with polytolerized (A, poly-tol) or hybrid-tolerized (B, hybrid-tol) mice

A) Tolerance induction with peptide mixture

		IgG1 (OD)	IgE (OD)	IgG2a (OD)
Bet v 1	poly-sens	1.77 ± 0.48	0.71 ± 0.47	0.46 ± 0.29
	poly-tol	1.91 ± 0.16	0.61 ± 0.48	0.93 ± 0.69
Phl p 1	poly-sens	1.92 ± 0.51	0.23 ± 0.09	0.31 ± 0.10
	poly-tol	1.57 ± 0.43	0.32 ± 0.21	1.02 ± 0.77
Phl p 5	poly-sens	1.12 ± 0.02	0.48 ± 0.29	0.90 ± 0.42
	poly-tol	1.37 ± 0.37	0.59 ± 0.39	1.57 ± 0.46

B) Tolerance induction with hybrid peptide

		IgG1 (OD)	IgE (OD)	IgG2a (OD)
Bet v 1	poly-sens	2.11 ± 0.33	0.90 ± 0.69	0.18 ± 0.06
	poly-tol	2.29 ± 0.11	1.10 ± 0.88	1.04 ± 0.89
Phl p 1	poly-sens	1.93 ± 0.58	0.41 ± 0.11	0.43 ± 0.32
	poly-tol	1.87 ± 0.60	0.42 ± 0.32	1.08 ± 0.82
Phl p 5	poly-sens	1.31 ± 0.19	1.35 ± 0.53	0.55 ± 0.32
	poly-tol	1.57 ± 0.51	0.55 ± 0.25	1.07 ± 0.60

[0091] Polysensitized: Average values (± SD) of five mice with a composition of Bet v 1/Phl p 1/Phl p 5 adsorbed on $Al(OH)_3$; Polytolerized: Average values (± SD) of five mice pretreated with a composition of the immunodominant peptides of Bet v 1, Phl p 1 and Phl p 5; Hybrid-tolerized: Average values (± SD) of five mice pretreated with a hybrid peptide; OD = Optical density.

[0092] Cytokine production in vitro: Both pretreatments led to a significant drop in IL-4 and IL-10 production in polytolerized animals in comparison with polysensitized control animals (Figure 3). In contrast, IFN-γ concentration increased considerably (Figure 3). After intranasal application of the peptide mixture (BP: polysensitization 46.29±21.11 pg/ml in comparison with polytolerance 12.45±8.10 pg/ml, $p<0.05$; Phleum extract: polysensitization 272.86±125-38 pg/ml in comparison with polytolerance 78.84±10.73 pg/ml, $p<0.05$), IL-5 production was considerably weaker in the mice, but not in mice pretreated with the hybrid peptide (BP: polysensitization 25.82±6.50 pg/ml in comparison with hybrid tolerance 17.24±12.62 pg/ml; Phleum extract: polysensitization 146.46±60.69 pg/ml in comparison with hybrid tolerance 178.63±101.25 pg/ml).

Figure 1: Simultaneous sensitization with the recombinants Bet v 1, Phl p 1 and Phl p 5 led to a comparable antibody response to all three allergens. This shows that we have set up a model for polysensitization in mice (cf. Fig. 1).

Figures 2A and 2B: Results of antibody concentration against the three allergens after intranasal tolerance induction of the peptide mixture (A) or the hybrid peptide (B). In both cases a decrease in antibody concentration was obtained (cf. Figs. 2A and 2B).

Figure 2A: Tolerance induction of peptide mixture

Figure 2B: Tolerance induction of hybrid peptide

Figure 3: Results of tolerance induction of peptide mixture (A) or hybrid peptide (B) on cytokine production. Interleukin 4 and interleukin 10 production was considerably reduced after treatment both with the peptide mixture and with the hybrid peptide (cf. Fig. 3).